

PREVALENCE OF *Salmonella* spp IN SOME ENVIRONMENTAL SAMPLES FROM SOME HOUSEHOLDS ENGAGED IN LIVESTOCK FARMING IN SOME PARTS OF ZARIA, NIGERIA.

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ABSTRACT

*Salmonella* outbreaks from contaminated water and non animal foods (e.g. produce) are increasingly reported. To address the environment as a potential source of pathogenic *Salmonella*, the prevalence of *Salmonella* in households engaged in livestock farming in Zaria, Kaduna State (Nigeria) was investigated. A total of 336 environmental samples comprising water, soil, manure and vegetable samples were collected from thirty households with livestock while three households without livestock were used as control. Sampling was done from March to September 2006 covering both wet and dry seasons. Seven *Salmonella* spp were obtained: 1(0.65%) during the dry season and 6(3.28%) during the wet season. *Salmonella* spp were detected from manure, water and vegetable samples. A prevalence rate of 2.08% was obtained for *Salmonella* spp in the study location. The rate of detection was higher in the wet season than in the dry season. The detection of this pathogen from the environment represents a potential health risk factor since man is always in continuous interaction with the environment.

Keywords; Prevalence, *Salmonella*, Environment, livestock.

INTRODUCTION

The potential contamination due to animal husbandry operations and the indiscriminate disposal of animal wastes and effluents from livestock applied as fertilizer for crop or silage production constitute an environmental and public health risk (Yang *et al.*, 2004).

Adding manure to the soil has agronomic benefits through the addition of plant nutrients (nitrogen, phosphorus and potassium) and organic matter (Gagliardi and Karns, 2002). . Despite the benefits from the use of organic manure, there is a potential health risk from manure into the food chain as a means of pathogen transfer. Nicholson *et al.*, (2000) in the review of issue of pathogens transfer into the food chain from manure applications to land, states that there is a lack of data on 'typical' levels of pathogen in animal manures.

The actual number of pathogens shed is important and this has been found to be affected by a number of factors such as animal age, diet, stress and season (Nicholson *et al.*, 2000). The probability of pathogens being available for transport at the soil surface is also likely to be influenced significantly by the duration and conditions of storage prior to land spreading.

Contaminated manure can contaminate the produce directly through its use as a soil fertilizer or indirectly through infiltration of irrigation water or water used to wash the produce (Doyle, 2000). Sources of microbial pathogens on fresh produce at the preharvest stage include faeces, irrigation water, inadequately composted manure, soil, air, animals and human handling (Buck *et al.*, 2003). Salmonellosis is a disease caused by members of the genus *Salmonella* (Nietfeld and Kennedy, 1999). It is an important cause of diarrheal illness in humans, causing approximately 1.4 million illness and 600 deaths annually in the United States (Brenner *et al.*, 2000). Much of what is known about the epidemiology of samonellosis comes from outbreak investigations. These investigations have determined that most human infections result from the ingestion of foods of animal origin that are contaminated with *Salmonella* species(Mead *et al.*,1999) being *Salmonella* of animal origin causes an intestinal infection, characterized by sudden onset of fever, myalgia, cephalagia, abdominal cramps, nausea and vomiting. There is increased risk of exposure among those who work in abattoirs, poultry processing plants and those in contact with animal and their products.

This work examines the role of livestock and its effluents as point source of *Salmonella*.

#### STUDY AREA

The study area covered were some households engaged in livestock farming in Samaru and Sabo in Sabon Gari L.G.A and Zaria city in Zaria L.G.A of Kaduna State. Sabon Gari local government area is a large urban set up with a population of 246,544 people (FGN Gazzet,2009).The local government is situated along longitude 8° and 9° and latitude 10° and 11° on the world map and is bounded on the east by Soba L.G.A and on the west by Giwa L.G.A on the north by Makarfi L.G.A and south by Zaria L.G.A.While, Zaria local government area has a population of about 277, 187 inhabitants (Population Census, 1991). It is situated on longitude 8° and latitude 9° and it is bounded on the east by Soba L.G.A, west by Giwa L.G.A, north by Sabon Gari L.G.A and on the South by Igabi L.G.A.

Livestock keeping is a common activity in most households in the area ranging from poultry, cow, goat, sheep and ram. The semi intensive and intensive livestock management systems are common while the extensive is rare. Most households had wells situated in their residential area from where water for domestic activities, drinking and water for the animals is obtained. Some households have tap, though tap water is not a constant source of water, wells are the most regularly used. However, some households obtain water from stream or river. A total of three hundred and thirty six samples were analyzed. Three cluster settlements of close proximity to the University of Study (Samaru, Sabon Gari and Zaria city) were randomly selected. Within the cluster, households with livestock were identified and ten households randomly selected from each cluster population.

#### SAMPLE COLLECTION

##### Water

Water samples were collected according to the procedure recommended by American Public Health Association (APHA, 1992). Water samples were collected from the available water sources in each household visited comprising of water for domestic activities, drinking and watering of animals. All samples were collected in sterile wide-mouthed, screw-capped 250ml glass bottle, packed in ice during transportation to the laboratory (Postgraduate Laboratory, Department of Microbiology, ABU, Zaria). Samples were analyzed within six hours of collection. From the thirty-three households visited a total of seventy six water samples were collected (thirteen water samples from Samaru, ten from Sabo and nine from Zaria city during the dry season; March-May, giving a total of thirty-two samples). A total of thirty eight water samples were collected during the rainy season; June- August (Ten from Sabo, twelve from Samaru, sixteen from Zaria city) and six water samples from households without livestock in both seasons.

##### Soil/Manure Samples

During the study period, a total of one hundred and seventy manure samples were collected from the households visited; eighty five were collected during the dry season and eighty five during the wet season. During the dry season, manure was collected from twenty storage sites while sixty five fresh manure samples were collected. In the wet season however, manure was collected from thirty storage sites while fifty five were fresh manure samples. A total of sixty six soil samples were collected during the study period (Ten from each of the three locations and three from control sites in both seasons). All samples were collected aseptically using ethanol-sterilized spatula. A 100g of sample was collected at the designated site at five different points, one from the centre and four different points, on the periphery and mixed together to obtain a representative sample. Samples were transported to the laboratory in sterile plastic bags and analysed within six hours of collection.

##### Vegetable Samples.

Vegetables available in the study sites during the study period and which were grown on soils where manure was applied as fertilizer were collected. Using 90% ethanol-sterilized scissors; vegetables were cut into sterile containers and taken to the laboratory. A total of twenty four vegetable samples were collected consisting fourteen from Samaru, six from Sabo and four from Zaria city. Analysis was done within six hours of collection.

## MATERIALS AND METHOD

*Salmonella* was isolated from the samples using Selenite F broth enrichment and incubation (Harvey and Price 1979). Ten millilitres of each water sample was combined with equal volume of sterile double strength Selenite F broth and incubated at 37°C for 24h followed by streaking for isolation on *Salmonella-Shigella* agar. Isolation from soil and manure samples was by preenrichment of one gram of sample in nine millilitres of peptone water followed by enrichment in Selenite F broth and incubation at 37°C for 24h. The washed and cut vegetable was preenriched in Selenite F broth and incubated at 37°C for 24h. After the enrichment methods, *Salmonella* was detected by plating on *Salmonella-Shigella* (SS) agar for 24h at 37°C. Mixed cultures were transferred to fresh SS agar plates and streaked out to obtain pure colonies. Typical *Salmonella* colonies which appear colourless with black centers were picked and independently confirmed by Gram staining. Isolates were stored on slants of nutrient agar after incubation at 37°C for 24h. The slants were presumptive *Salmonella* isolates.

All the presumptive isolates were subjected to series of biochemical tests (indole, methyl red, citrate, Voges Proskauer, triple sugar iron, motility and oxidase test.). Biochemical identification of isolates was as described by Singleton (1997) and Farmer (1999).

Isolates suspected to be *Salmonella* were serologically tested using *Salmonella* polyvalent 'O' group A-Z antiserum latex kit according to the instructions of the manufacturer (Oxoid) Basingstoke, Hampshire, England). All isolates were streaked on blood agar plates and incubated at 37°C for 24h. Latex reagents were brought to room temperature, one drop of the test latex was dispensed onto a circle on the reaction card and a drop of saline was placed on the circle distant from the latex. Using a loop, a portion of the colony of presumptive *Salmonella spp* on blood agar plates was emulsified in the saline drop on a portion of the circle on the card. The test latex and the resulting smooth suspension were mixed together and spread to cover the reaction area using the loop. The card was rocked in a circular motion observing for agglutination within one minute. Agglutination of the test latex within one minute was recorded as positive result.

The association between the detection of pathogens and location was assessed using chi-square test of association and analysis of variance (ANOVA) was used for pair wise multiple comparison of mean counts from the three locations.

## RESULTS

Analysis of questionnaire showed that the intensive (confined) and semi intensive (allowed to move around) livestock management systems were predominant in the study area. Of the thirty households with livestock visited, 16 (53.3%) practiced the intensive management system 5 (31.3%) in Sabo, 6 (37.5%) in Samaru and 5 (31.3%) in Zaria city. The remaining households 14 (6.7%) practiced semi intensive management system; 6 (42.9%) in Sabo, 4 (28.6 %) in Samaru and 4 (28.6%) in Zaria city. Among the households visited, 10 (33.3%) own poultry, 8 (26.7%) own cows, 10 (33.3%) own goats and 2 (6.7%) own sheep. On the method of manure disposal, 19 (63.3%) dispose manure in the bush around the house, 5 (16.7%) dispose manure on the farm, 5 (16.7%) dispose manure near the farm and 1 (3.3%) dispose theirs just beside the house in heaps. In response to domestic water source, 22 (73.3%) obtain water from wells, 6 (20%) from tap and 2 (6.7%) obtain water from river/stream. Among the households visited, only 8 (26.7%) had vegetable farm and use manure to fertilize them.

A total of 336 environmental samples (water, soil, manure and vegetables) were analyzed to detect the presence of *Salmonella spp*. Table 1 shows that 104 (30.95%) samples were collected from Sabo, 107 (31.85%) from Zaria city and 125 (37.20%) from Samaru.

Sixty five isolates of *Samonella spp* were obtained. Twenty three (35.38%) were presumptive for *Salmonella spp*. and 7(30.43%) were confirmed *Salmonella spp* against *Salmonella* antiserum. Two out of 104 samples from Sabo, four out of 125 samples from Samaru and one out of 107 samples from Zaria city were positive for *Salmonella spp* (Table 1).

**TABLE 1** Samples containing pathogens in the locations studied

Location	Source of samples	Total no of samples	<i>Salmonella</i> isolates
<b>Sabo</b>	Water	26	-
	Soil	22	-
	Manure	50	2
	Vegetables	6	-
Sub total		<b>104</b>	<b>2</b>
<b>Samaru</b>	Water	25	2
	Soil	22	-
	Manure	64	2
	Vegetables	14	-
Subtotal		<b>125</b>	<b>4</b>
<b>Zaria city</b>	Water	25	-
	Soil	22	-
	Manure	56	-
	Vegetables	4	1
Subtotal		<b>107</b>	<b>1</b>
Overall total		<b>336</b>	<b>7(2.08)</b>

Key = Pathogen not detected from samples. Isolation Frequency =  $\frac{\text{No of samples containing pathogens}}{\text{No of samples analysed}}$

**TABLE 2** Prevalence of pathogens in environmental samples by season

Pathogens by Season	Location			Total	Isolation frequency of pathogens (%)
	Sabo	Samaru	Zaria city		
Salmonella spp					
D153	-	1	-	1	1(0.65)
W183	2	3	1	6	6(3.28)

Key

- = pathogen not detected from samples.

D=Dry season

W=Wet season

N=Number of samples collected

*Salmonella* is reported to have a low infective dose of 10 cells (Wall *et al.*, 1994). In this study, the isolation rate of 2.08% (7 in 336 samples) for *Salmonella* is therefore significant. Case control studies of sporadic *Salmonella* infections have implicated raw or under cooked eggs (Delarocque *et al.*, 1998) and to a lesser extent, chicken (Kimura *et al.*, 1998) as vehicles for transmission. Remarkably, in this study (57%) of the seven *Salmonella* spp were isolated from chicken manure. The isolation of *Salmonella* from water and vegetables treated with manure in this study supports the reports of Angulo *et al.*, (1997) that there are other vehicles, including non animal foods such as fresh fruits and vegetables. The presence of pathogenic bacteria, viruses and parasites on fresh fruits and vegetables has been extensively documented (Beuchat, 1996).

The isolation frequency for *Salmonella* (2.08%) in this study is lower than the 13.7% isolation frequency recorded by Kwaga *et al.*, (1985). The lower frequency seen in this study may be as a result of changes in the incidence of *Salmonella* in Zaria. Studies showed that salmonellosis demonstrates a marked pattern of seasonality increasing in the warm summer months as in other pathogens such as *E. coli* O157:H7 (Slutster *et al.*, 1997). This may be the reason for the isolation of *Salmonella* between the months of April and August in this study. However, the reasons for this pattern are not entirely known. It may be related to infection trends in animal hosts or to temperature change and warm summer months.

The inability to isolate *Salmonella* from soil samples could be due to competition with other soil microorganisms and adverse environmental conditions. The survival in soil of pathogens originating from animal manure and sewage sludge has been reviewed by Nicholson *et al.*, (2000) and a number of environmental factors have been shown to influence the survival of micro-organisms in soil (e.g. temperature, moisture content, sunlight, pH and the availability of organic matter). These reviewers appear to agree that temperature is the most significant and survival time increases with decreasing temperature.

Agricultural practices and livestock management systems should be modified, close proximity of animals to man's immediate environment should be avoided. Appropriate handling of manure is necessary to control spread of pathogens and to limit the risk of human infection. It may be necessary to hold manure for extended periods prior to spreading on farmland, or for use in the production of food crops, particularly foods that are to be consumed in the raw or minimally processed state. Application of processes such as composting, heat drying or digestion which can expedite the decline of pathogens is important.

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